REMARKS

The Examiner has expressed a concern that the Declaration of record provides data generated by non-sequence based probe separation techniques, but that such is not in line with the teachings of the specification, pointing in particular to page 13 of the specification.

Applicants maintain that the specification sets forth two different embodiments, as discussed in detail at page 14 of the Amendment After Final filed September 21, 2007. Namely, the first embodiment of the present invention relates to the use of samples, particularly blood, which are distant to the area of disease, e.g., a tumor, for diagnostic purposes, and the advantages that ensue from that invention, see page 10, last two paragraphs, page 23, second full paragraph and page 15, lines 11-14 of the present specification. The second embodiment concerns non-sequence based techniques, as described on page 13, lines 20 *et seq* of the present specification, and this embodiment also has particular advantages.

The Examiner believes that the use of non-sequence based techniques must be read into the entire teaching of the specification. However, Applicants respectfully submit that the specification provides clear evidence that this is not the case.

More specifically, on page 4, lines 11 *et seq* of the specification, ways of putting the invention into practical use are described. Reference is made to preparing standard and

patient specific patterns (SDPPs and PSPPs). Starting four lines from the bottom of page 4 it is stated:

> To design and develop the pattern for SDPPs, characteristic for one ailment, known techniques of isolation of mRNA, construction and amplification of cDNA and selection through differential hybridisation and differential display may be used. (Emphasis added)

Thus, the specification specifically teaches that selection may be performed through differential hybridization and differential display.

Differential display is a technique which was invented in 1992 by Drs. Arthur Pardee and Peng Liang to allow detection of altered gene expression. The Differential Display technology works by resolution of the nucleic acid fragments of interest on a gel. This allows direct side-by-side comparison of the nucleic acid species from different samples. The Differential Display method thus, allows one to visualize all of the expressed genes in the sample in a sequence-dependent manner. The method enables the recovery of sequence information and the development of probes to isolate their cDNA and genomic DNA for further molecular and functional characterizations. This method thus, provides the "non-sequence based" methods which are discussed in the application and exemplified in, for example, Example 4 on page 31, paragraph 4.1.8.

However, as noted above, the specification also explicitly refers to the alternative method of differential hybridization. As its name suggests, this method involves hybridization and thus, necessarily concerns sequence based separation techniques for probe selection. See: Calvet, *Pediatr. Nephrol.*, 5:751-757

(1991); a copy of which is attached hereto, which discusses differential gene expression and in particular differential screening involving hybridization (known as differential hybridization), see page 754, left hand column. In this method, a library of sequences is probed with labelled probes from two different samples which hybridize to the library of sequences. The differential expression evident between the different samples allows the identification of differentially expressed transcripts. This method is explicitly based on sequence specific hybridization between probes and the sequences to which they bind.

The above methods have been routinely used in the art to identify differentially expressed genes. See: Gress et al, *Oncogene*, 13:1819-1830 (1996); a copy of which is attached hereto, in which differential expression of genes in pancreatic cancer tissue was examined. In the methods that were used, cDNA libraries were prepared from pancreatic cell lines and cancer tissues. 20,000 cDNA clones were randomly picked and spotted onto membranes. Total RNA was isolated from control and pancreatic cancer tissue samples, probes generated and hybridized to the library clones (see page 1828, left hand side, first two paragraphs). The results are shown, for example, in Figure 1. From this study, 408 cDNA clones were classified as preferentially expressed in pancreatic cancer tissues and these were selected (see page 1820, right hand column, under the heading "Characterization of a pancreatic cancer-specific expression profile").

See also: Shalon et al, *Genome Research*, 6:639-645 (1996); a copy of which is attached hereto, which similarly describes a differential hybridization technique in which microscopic arrays of DNA fragments are immobilized on glass substrates to which yeast chromosomes are hybridized to identify the relative abundance of specific sequences in the samples (see the abstract and the results in Figure 1).

Thus, differential hybridization techniques were well-known in the art at the time of the invention for identifying transcripts, and hence their genes which are differentially expressed under particular conditions. Such techniques are clearly "sequence-based" relying on the particular sequence of the transcripts to bind to relevant capture sequences to determine which transcripts are less or more abundantly expressed. Such methods are clearly and specifically taught in the specification and thus, clearly the use of "sequence-based" methods for probe separation and isolation is taught. This is in line with Applicants arguments that two different inventions are taught in the present specification.

This is further born out by the Examples in the present specification. Example 6 describes experiments that have been performed in accordance with the invention. It should be noted that the first step on page 38, refers to attaching the informative probes to a nylon membrane for interrogation with cDNA generated from RNA from different samples. It is stated in step 1 that

> Most of the clones were isolated from a subtracted cDNA library of Norway spruce....(in accordance with the method described in Example 4).

Thus, the cDNA library subject to the method of differential display described in Example 4 is a subtracted cDNA library. Subtracted libraries are prepared by sequence-based methods which are used to enrich a library for the desired differentially expressed sequences. In such methods, single stranded cDNAs are synthesized and then hybridized to mRNA from the sample for comparison. The cDNA sequences representing mRNAs expressed in common will hybridize while those that are unique to either sample will remain single-stranded. The hybrids can then be removed leaving the enriched differentially expressed cDNA molecules. This method is described in Calvet, *supra*, on page 754, right hand column.

Thus, the method used in Example 6 clearly uses a preliminary "sequence-based" enrichment method prior to differential display. Example 6 is concerned with methods in which systemic effects are observed distant to the site of infection, i.e., the first embodiment of the invention noted above. Thus, the present invention is clearly embodied by methods in which "sequence-based" separation and selection techniques are used, and this is generically taught at page 4 of the specification, which specifically refers to using known techniques which include sequence based separation and selection techniques of differential hybridization.

The Declaration of record uses differential hybridization to separate and select probes which are informative for identifying breast cancer. In paragraph 8 thereof, it should be noted that cDNA probes from test samples were hybridized to high density arrays carrying 32,878 oligonucleotides.

Differential display allowed the identification of information probes which were then used in diagnostic methods of the invention. Thus, the methods as used are entirely in accordance with the teaching of the specification which teaches the use of differential hybridization for probe selection.

It should be noted that the methods used to derive the probes will not substantially affect the results. The different methods which can be used will identify transcripts which are up or down regulated in disease. It is Applicants' finding that when used in combination, many probes identified in any way can be used for diagnostic purposes. The present invention is based on the fact that the systemic effects of disease on the body and in the present case, on blood cells, subtlety or significantly affects the transcription levels of many genes. Thus, by using several probes and examining the expression of the corresponding genes, a "fingerprint" can be developed which is diagnostic of the disease condition.

As a consequence, different techniques can be used, with success, to identify probes of interest. A full cDNA library may be screened to identify probes, or a selection of randomly picked molecules or a subtracted library may be screened. Differential display or differential hybridization techniques may be used. Combinations of these techniques may be used. Results generated by these techniques will not be substantially different and will all support the present invention in issue, namely the use of blood samples to identify differentially expressed genes which can be used to generate probes for diagnosis of breast cancer.

In view of the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at the below-listed number on any matters which might arise.

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